T Cell Turnover in SIV Infection

In their report of 20 February 1998 (*1*), H. Mohri *et al*. analyze the proliferation and death of T cells in normal macaques and in those infected with simian immunodeficiency virus (SIV). With the use of bromodeoxyuridine (BrdU) to label proliferating cells, they observed that the uptake of BrdU by both $CD4^+$ and $CD8^+$ T cells was as much as threefold faster in infected than in uninfected macaques during the 3-week labeling period. After cessation of BrdU administration, the fraction of labeled cells gradually decreased, which indicated that labeled cells were dying more rapidly than they were proliferating. Because Mohri *et al.* assume that the labeled cells were indicative of the entire pool of T cells that acquired the label during the experiment, and that the T cell population as a whole was essentially at steady state during the study, they conclude that the difference between the rates of death and proliferation must be compensated for by a supply of new T cells ("replacement") to the pool, possibly from the thymus. Mohri *et al.* use a mathematical model and adjust its parameters—the various rates of death, proliferation, and replacement—to fit the kinetic data.

One of the assumptions in this model is that all T cells in the pool, labeled and unlabeled alike, are uniformly proliferating at some constant rate and dying at a higher constant rate [see figure 2 in the report (*1*)]). This assumption is incorrect. It is quite likely that many (probably most) of the labeled T cells in infected macaques had divided in response to immunologic stimulation by their cognate antigen, or by induced cytokines, or by both. It is now well known that such activated cell populations initially expand; the great majority of cells in these populations then die (mainly by apoptosis mediated by fas and fas ligand). Other activated T cells may be converted into "resting" memory cells, or enter a state of anergy in which their proliferation ceases although the cells survive. Thus, it is quite likely that Mohri *et al.* were mainly tracking the short-term expansion (during labeling) and contraction (during delabeling) of immunologically stimulated cell populations, rather than a stationary turnover of the entire T cell population. When BrdU was no longer administered, peripheral T cells other than those that had earlier been labeled were preferentially activated, in response to new stimuli. The preferential expansion of unlabeled cells is a more likely explanation for the washout dilution of labeled cells than is an influx of new cells from the thymus or some other source. In uninfected macaques, T cell proliferation may be associated to a larger degree with physiologic regeneration and less with immune activation, so that the labeled cells would be more representative of the population as a whole. Indeed, in some of these animals there was almost no delabeling [figure 1 in (*1*)].

A simple model (Fig. 1) of normal, ongoing immune activation (*2*) can account (Fig. 2) for the rapid rise in the fraction of labeled cells during the labeling period and for the rapid decline thereafter (*3*). A crucial aspect of our model is that the processes of T cell regeneration, expansion, and death are largely associated with different sequential compartments (stages of activation and maturation). When labeling of dividing cells occurs for a finite length of time, sufficient only for a fraction of the cells to become labeled, cells in the activated compartments are disproportionally labeled as compared with "resting" cells. The "growing-while-aging" structure of activated cells (Fig. 1) implies that the selected cohort of labeled cells must be essentially transitory. This transitory aspect should not be interpreted to reflect unbalanced overall birth and death rates in the T cell population as a whole or even in the activated cell population (*4*).

Increased immunologic stimulation in infected individuals most likely accounts for "increased turnover" rates in both CD4 and CD8 cells. Mohri *et al.* (*1*) mention some well-documented manifestations of immune activation (p. 1226), but their model (pp. 1223–1224) describes only regenerative proliferation, in which all the cells have the same proliferative capacity, which remains constant over time. This led Mohri *et al*. to ask questions about the mechanism responsible for the apparent "killing" of both CD4 and CD8 lymphocytes in infected individuals, in excess of proliferation, and about the differential depletion of CD4 cells (*1*, p. 1226):

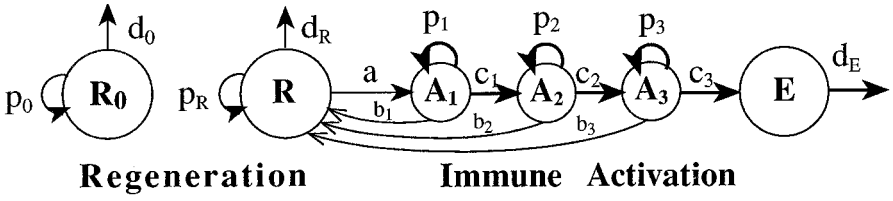

Fig. 1. Schematic representation of T cell regeneration and activation in our model. Variables (circled) and parameters are defined in (*2*).

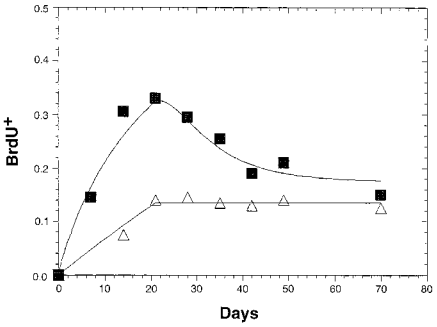

Fig. 2. Changes in the fraction of BrdU-labeled $CD4^+$ T cells during 3 weeks of labeling and 7 more weeks of no labeling [data from Mohri *et al*. (*1*)]. Measurements shown are from two representative macaques: (■), data from an infected macaque; (△), from uninfected macaque. Curves were generated with the use of our mathematical model; details in (*3*).

"Why then is CD4 T cell depletion observed and not CD8 T cell depletion . . . ?" But if our interpretation is correct, the death of immune activated cells serves to control their earlier accelerated proliferation and need not affect the equilibrium condition of the population as a whole. Accordingly, the death of labeled cells has little bearing on the issue of depletion.

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References and Notes

1. H. Mohri, S. Bonhoeffer, S. Monard, A. S. Perelson, D. D. Ho, *Science* **279**, 1223 (1998).
2. In Fig. 1, R represents the pool of resting T cells (either $CD4^+$ or $CD8^+$ cells) that are asynchronously activated (specifically or as "bystanders") at an average rate, a, over the time of the experiment. The pool regenerates at a rate p_R; the death rate is d_R. R_0 denotes the pool of resting memory and naïve T cells that are not subject to immune activation during the time of the experiment. (The partition into R and R_0 is not essential for the present simulation. It becomes more relevant when the turnover of cells with particular phenotypes, related to the cells' activation history, is being considered.) A_1, A_2, and A_3 are activated cells at different states of activation and maturation (the partition into three classes is arbitrary). c_i (i = 1, 2, or 3) denote the maturation rates, p_i the proliferation rates, and b_i are the back-flow rates of memory cells returning to the resting compartment. E are nondividing, fully activated ("effector") cells that die at a rate d_E. (More generally, cells in E may also divide and give rise to memory cells at some

rates.) The system is assumed to be at steady state, leading to the following conditions: $F_R = (p_R - d_R - a)_R + b_1A_1 + b_2A_2 + b_3A_3 = 0$; $F_1 = (p_1 - c_1 - b_1)A_1 + aR = 0$; $F_2 = (p_2 - c_2 - b_2)A_2 + c_1A_1 = 0$; $F_3 = (p_3 - c_3 - b_3)A_3 + c_2A_2 = 0$; $c_3A_3 - d_EE = 0$; $p_0 = d_0$. The equations for the labeled cells in the different compartments are $dR^L/dt = F_R{}^L + 2p_R(R - R^L)S_{21}$; $dA_1{}^L/dt = F_1{}^L + 2p_1(A_1 - A_1{}^L)S_{21}$; $dA_2{}^L/dt = F_2{}^L + 2p_2(A_2 - A_2{}^L)S_{21}$; $dA_3{}^L/dt = F_3{}^L + 2p_3(A_3 - A_3{}^L)S_{21}$; $dE^L/dt = c_3A_3{}^L - d_EE^L$; $dR_0{}^L/dt = 2p_0(R_0 - R_0{}^L)S_{21}$. Here $F_i{}^L$ is the same as F_i ($i = R,1, 2,$ or 3), with the variables on the right-hand side of F_i replaced with their labeled counterparts. S is a step-function of time t: $S_{21} = 1$ if t is smaller than, or equal to, 21 days; $S_{21} = 0$ for $t > 21$. S allows acquisition of BrdU by unlabeled cells to occur during the first 3 weeks, but not later.

3. The equations listed in (*2*) were used as shown in Fig. 2 to fit data reproduced from the report by Mohri *et al*. [figure 1A, upper-left panel in (*1*)]: the changes in fraction of cells that are $BrdU^+$ in the $CD4^+$ lymphocyte subpopulation of a highly infected macaque (square points) and of an uninfected macaque (triangles) [animals # RH-1316 and # RH-1372, respectively, in (*1*)]. Mohri *et al*. also used their model to fit CD4-turnover data from a few other macaques, but these two examples are representative. In Fig. 2 of our comment, the smooth curves demonstrate consistency of our equations with the data after fitting the parameters. For the uninfected animal the data are compatible with $R = 0$ (no activation) and $p_0 = d0 = 0.0035$ day^{-1}. The set of parameters that fit the labeling-delabeling kinetics in the infected macaque is not unique, and we have made no effort to optimize our choice, either biologically or numerically. The set used in Fig. 2 is $R_0 = 0.5$ (half the total number); $p_R = 0.002$, $d_R = 0.0035$, $a = 0.0044$, $p_1 = p_2 = p_3 = 1$, $c_1 = 3.9$, $c_2 = 1.5$, $c_3 = 1.1$, $b_1 = b_2 = b_3 = 0.07$, $d_E = 0.1$, $p_0 = d_0 = 0.0035$ day^{-1}. We have assumed, for simplicity, the turnover of nonactivated CD4 cells in the infected macaque to be the same as that in the uninfected macaque. This may well be an overestimate: As we have argued in the past, chronically elevated immune activation may actually lead to inhibition of regenerative proliferation in the resting cell population (*5*). The size of this population might consequently be reduced. On the other hand, the total number of all other cells belonging to the same T cell subset may increase or decrease, depending on the average expansion of activated clones and on the rate of memory cell back-flow relative to the activation rate of resting cells. These considerations underscore the complexity of the relationship between immune activation and homeostasis (*5*).
4. In note 11 in (*1*), Mohri *et al*. indicate that "Although the source may represent cells being exported from the thymus or extrathymic tissues, . . . it could also include a population of resting or slowly dividing T cells, which upon activation would undergo rapid clonal expansion and enter the subpopulation of cells acquiring label during the experiment." However, "resting T cells" are themselves dynamic part of the population of cells acquiring label, during their regeneration and also through the conversion of immune activated cells into resting memory cells. Furthermore, the expansion of immune-activated cells alone, irrespective of the "source," more than compensates for their subsequent contraction. Outflow of memory cells from the activated pool into the resting pool may actually exceed the inflow of activated cells. Thus, although activated clones continuously replace each other, the activation of resting cells can hardly be seen as a homeostatic mechanism in SIV-infected macaques, or in HIV-infected humans.
5. Z. Grossman and R. B. Herberman, *Nature Med.* **3**, 486 (1997).
6. We thank W. E. Paul (NIH) for his substantial contribution to this communication and S. J. Merrill (Marquette University) and I. Rouzine (Tufts University) for helpful discussions of mathematical issues. Supported in part by grant 93-00339 from the United States–Israel Science Foundation, Jerusalem, Israel.

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Mohri *et al*. (*1*) studied turnover of T cells in SIV-infected macaques by adding BrdU to drinking water of the animals. If their interpretation of BrdU labeling kinetics is correct, then rapid direct or indirect killing of T cells by SIV is compensated, to a large extent, not by proliferation of other T cells, but through supply of new cells from an as-yet-undefined source.

At issue is the explanation of the high rate of loss of BrdU-positive cells after the labeling has stopped. At that stage, unlabeled cells must divide into unlabeled cells, and labeled cells will generate labeled progeny. Given the quasi-steady state that characterizes SIV-infected animals, if proliferation of T cells almost fully compensates for their loss, then rather than decreasing rapidly, as observed, the fraction of BrdU-positive cells should have remained almost constant until the label per cell becomes diluted to a point below the limit of detection, a period much longer than the cell turnover time. To account for this paradox, Mohri *et al*. postulate that replenishment of T cells is partially provided by a source that has labeled and unlabeled components [equations 1 and 2 in (*1*), pp. 1223 and 1224], whose relative intensities are constant during the labeling phase [note 11 in (*1*), p. 1227], and that the labeled source component suddenly becomes very small when external labeling has stopped ($S_L' = 0$ to obtain equation 3, p. 1224; note 14, p. 1227). Mohri *et al*. do not define a biologically consistent model of a source with such hypothetical properties (note 11, p. 1227), although this was a central point in the interpretation of their experiments.

We propose an alternative and simpler explanation, based on dilution of the BrdU label which will occur, according to a model of T cell replenishment (*2*), as follows. A resting T cell in vivo, on receiving an activation signal, may go through many cycles of division before resuming the resting state. For example, six cycles, each of 1 day or less, would be sufficient to dilute the label per cell below the gate value within 1 week. (Immune activation of T cells in vitro offers a biological example of such proliferation mechanism. The proliferation loop may involve additional cell types, such as effector cells.) In the quasi-steady state, such multiple, rapid proliferation bursts should be compensated by the average rate of death of activated primed T cells. When a labeled cell becomes activated during (or shortly before) the follow-up period after labeling has stopped, this cell and its progeny necessarily "disappear" (become undetectable) within a week, and are replaced, on average, by an unlabeled cell. As a result, the fraction of labeled cells will decay on a time scale of a few weeks, according to the estimated activation rate that can be inferred from the rate of labeling during the labeling phase.

Mohri *et al*. have argued against this model (loss of labeled cells resulting from dilution of label) as an explanation for the observed decline in the labeled fraction because their "BrdU-intensity data did not reveal any substantially lower values with the passage of time" [note 13 in (*1*), p. 1227]. (A slow decline in the BrdU amount per labeled cell would be expected in the one-division model used by Mohri *et al*.) In fact, the multiple rapid division model, although based on dilution, does not predict a gradual decline in the amount of BrdU per cell. Instead, the fraction of labeled cells activated within a time interval is predicted to fall swiftly (within a few days) below the BrdU intensity gate value. Such a fast process is difficult to detect, especially with the wide sampling interval of 1 week used by Mohri *et al*. The level of BrdU intensity among labeled cells that are not activated yet will always remain high, but the number of such cells will decline according to the activation rate.

We offer these considerations in the hope of encouraging further studies into the nature of the enhanced turnover of different lymphoid cells imposed by SIV in infected animals and by HIV in humans.

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References

1. H. Mohri, S. Bonhoeffer, S. Monard, A. S. Perelson, D. D. Ho, *Science* **279**, 1223 (1998).
2. I. M. Rouzine and J. M. Coffin, in *Origin and Evolution of Viruses*, E. Domingo, R. Webster, J. Holland, Eds. (Academic Press, London, in press).
3. We thank Z. Grossman for helpful discussions.

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Response: We have shown that after BrdU is administered to SIV-infected or healthy macaques for 3 weeks, a population of BrdU-labeled cells is created, which slowly decays (*1*). Because the progeny of BrdU-labeled cells should remain labeled until a sufficiently large number of divisions have occurred to dilute out the label, we interpreted this loss as suggesting that the subpopulation of cells that were labeled during the 3 weeks of BrdU administration died more rapidly than they were produced. This further implied that in steady state there must be a source of cells that enter this subpopulation.

Grossman *et al*. and Rouzine and Coffin both state that the subpopulation we studied consists of cells stimulated, possibly by antigen, SIV, or induced cytokines, into rapid division, and which would die rapidly (that is, by Fas/Fas-ligand mediated apoptosis). Although such events undoubtedly occur, the question is whether they are the dominant

population or only a subpopulation of the cells under study. Other experiments will be required to answer this definitively, but our impression is that if the subpopulation that we tracked were dominated by cells undergoing short-term antigen-stimulated expansion and contraction, with division rates of a day or less (Rouzine and Coffin), then it is difficult to see why the fraction of labeled cells graduately increases over the 3-week labeling period rather than going through spikes and dips. Further, over the 3-week labeling period, as many as 30 to 40% of $CD4^+$ T cells label in some SIV-infected animals. Because the antigen specificity of these cells was not determined, we cannot address the question of whether these cells derive from a few stimulated clones or are broadly representative of the $CD4^+$ T cells in the animals. But no illness was observed in the animals and no immunizations were performed. Moreover, immune stimulated cells tend to die rapidly, yet even 7 weeks after labeling has stopped, labeling cells still exist and their decay remains on the same straight line in a semi-logarithmic plot. The rate of delabeling, according to our model is $d - p$. Because p is generally small, our fitting procedure is unlikely to greatly underestimate the death rate. On the basis of the slope of delabeling, the average death rates of both infected and healthy monkeys correspond to cell half-lives of 2 weeks or more, not the few days required by the scenarios of Grossman *et al*. and Rouzine and Coffin. In some instances, BrdU is toxic to cells. If some toxicity were present, then the natural half-lives would be even longer.

The model proposed by Grossman *et al*. has several shortcomings. First, for the chosen parameters, the total cell population in infected animals is not in steady state, but in a slow but unbounded exponential growth phase. Second, in uninfected animals, the resting but activatable cell population (denoted by R in their model) would disappear in the long term, since their death rate exceeds their proliferation rate. Grossman *et al*. appear to misinterpret our model when they say that we assume that all T cells in the pool are uniformly proliferating at a constant rate. Our model describes the mean behavior of a population of cells. Thus, individual cells can proliferate with different rates, or not at all. Our proliferation rate, p, and the death rate, d, are meant to be the average proliferation and death rates, respectively, of the $CD4^+$ cells measured in the periphery. Modeling in this way has the advantage of introducing only a small number of parameters, as opposed to the 15 parameters used in the model by Grossman *et al*.

Grossman *et al*. suggest that the turnover that we have measured has little bearing on the issue of $CD4^+$ T cell depletion in HIV or SIV infection. We disagree, and we show (Fig. 1) new data collected at 22 weeks, that is, 19 weeks after the stop of labeling, of our original experiment (*1*). The percentage of $CD4^+$ cells that were $BrdU^+$ at 10 weeks that are still $BrdU^+$ at 22 weeks versus baseline CD4 count for the monkeys we studied are shown (Fig. 1). Monkeys with low CD4 count (that is, SIV-infected ones) have greatly enhanced loss of labeled CD4 cells as compared with monkeys with high CD4 counts (uninfected ones). If this loss is a result of label dilution, as argued by Rouzine and Coffin, then these results suggest that SIV infection causes enhanced proliferation, whereas if the loss is a result of the rate of cell death being greater than the rate of proliferation, as in our model, then the results show increased death. In either event, the biological conclusion is that SIV infection causes enhanced $CD4^+$ T cell turnover.

In summary, we agree with both comments that immune stimulation in SIV infection may be elevated and may be the cause of the increased proliferation of all of the various lymphoid populations that we measured. However, because as many as 30 to 40% of T cells are labeled after 3 weeks and no antigen was intentionally given to stimulate a response, we believe that the majority of cells we monitored are representative of the general T cell population, although a minority may be antigen-activated. As explained in reference note 11 of our report (*1*), our model is consistent with the possibility that the source for cells moving into the subpopulation under study could include a population of resting or slowly dividing T cells, which upon activation would undergo rapid clonal expansion and acquire label (*4*). Thus, the clonal expansion that Grossman *et al*. and Rouzine and Coffin are concerned about may be the source. The input of cells that we described from the "source" corresponded to a 1% replacement rate in uninfected animals and 2.5% in highly infected animals, percentages that are consistent with Ki67 labeling and other measures of immune activation that suggest ~1% lymphocytes are activated at a given time in healthy individuals, increasing three-fold during HIV-1 infection (*2–3*).

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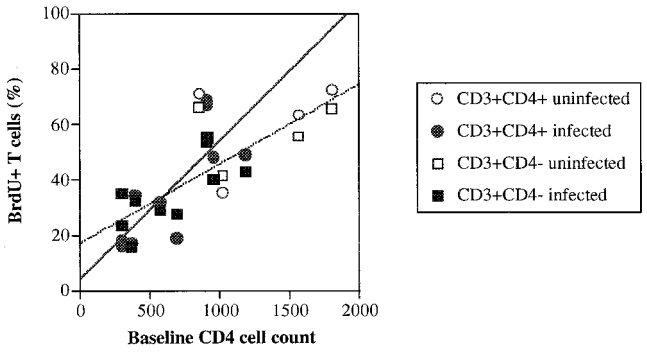

Fig. 1. Decline of $BrdU^+$ cells during week 10 and 22 correlates with the baseline of CD4 cell count. Data points indicate the percentage of CD4 and CD8 cells that were $BrdU^+$ at week 10 that are still $BrdU^+$ at week 22. Correlation of the decline of $BrdU^+$ cells versus the baseline CD4 cell count are shown. Solid line and dot line are the curve fitting for the data of $CD3^+CD4^+$ cells and $CD3^+CD4^-$ cells only from infected monkeys.

References and Notes

1. H. Mohri, S. Bonhoeffer, S. Monard, A. S. Perelson, D. D. Ho, *Science* **279**, 1223 (1998).
2. Z.-Q. Zhang *et al*., *Proc. Natl. Acad. Sci. U.S.A.* **95**, 1154 (1998).
3. N. Sachsenberg *et al*., *J. Exp. Med.* **187**, 1295 (1998).
4. To explicitly show how cell activation influences our published model, consider two populations of cells: a slow population, *S*, which divides infrequently or not at all during the 3-week labeling period, and a fast population, *F*, which divides frequently. The simplest model for the slow population is one in which the population is self-sustaining—that is,

$$dS/dt = (p' - d' - a')S$$

where p', d' and a' are the per cell rates at which *S* slows proliferate, die and become activated, respectively. However, equivalent results can be obtained if the population has a source, λ, rather than proliferative self-renewal, that is, $dS/dt = \lambda + (p' - d')S$. In any event, assume that the size of the S population remains constant so that $dS/dt = 0$. The resting or slowly dividing population, upon activation, is now assumed to clonally expand very rapidly, into a clone of *F* cells of average burst size *c*. Thus,

$$dF/dt = aS + (p - d)F$$

where $a = ca'$ and p and d are the per cell proliferation and death rates of F cells. Now provide BrdU. In the simplest case, S cells divide so infrequently that they do not label and the number entering the population, say from the thymus, is so small that it adds negligibly to the labeled pool. However, in the pres-

ence of BrdU, the F population subdivides into labeled and unlabeled cells, F_L and F_U, respectively, as clonally expanded cells are labeled during their expansion. Thus, when BrdU is present,

$$dF_U/dt = -(p + d)F_U,$$

$$dF_L/dt = 2pF_U + (p - d)F_L + aS.$$

If the total population of cells is constant, one can easily show that the fraction of labeled cells, f_L, obeys the equation

$$f_L(t) = C(1 - e^{-(p+d)t}) \quad (1)$$

where $C = F_U(0)/(F_U(0) + S)$. During delabeling,

$$dF_U/dt = (p - d)F_U + aS$$

$$dF_L/dt = (p - d)F_L$$

with solution

$$f_L(t) = C(1 - e^{-(p+d)T})e^{-(d-p)(t-T)} \quad (2)$$

where T is the time delabeling begins. Equations (1) and (2) are identical to the equation given in Mohri *et al*. (*1*) and establish that the source can be the clonal expansion of resting cells. A more detailed analysis of this model and its variants in which the slow cells divide and acquire label has been submitted elsewhere (*5*).

5. S. Bonhoeffer *et al*., in preparation.

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